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Comparative Bioinformatics Analysis of Melatonin Receptor in Human, Rat, and Fish

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Abstract **Original Research Article**

Melatonin is an indoleamine hormone primarily produced by the pineal gland. It plays a vital role in regulating circadian rhythms, sleep–wake cycles, and several physiological functions, including antioxidant defense and immune response. Its effects are mediated through G protein–coupled receptors, mainly MT1 and MT2, which show structural and functional differences across species. To conduct this analysis, several bioinformatics tools were used: receptor sequences were retrieved from NCBI in FASTA format; interaction profiles and binding affinities were assessed using the STRING database; 3D structural models were predicted with Phyre2; and molecular visualizations were evaluated using Biovia Discovery Studio (BDS). This study presents a comparative bioinformatics analysis of melatonin–receptor interactions in humans, rats, and fish. The aim is to investigate evolutionary conservation and species-specific variations in receptor structure and ligand binding, which may affect receptor sensitivity and downstream signaling pathways. The results revealed distinct differences in binding site residues and interaction energies among the species, indicating evolutionary adaptations in receptor function. These findings offer valuable insights into the molecular diversity of melatonin signaling and may support the development of species-specific receptor-targeted therapeutics.

Keywords: Bioinformatics, Fish, Human, Melatonin, Rat, Receptor.

1. INTRODUCTION

1.1 Melatonin: Synthesis, Function, and Biological Role

Melatonin (5-methoxy-N-acetyltryptamine) is a neurohormone produced by the pineal gland, a small endocrine structure located deep in the brain. Its synthesis is regulated by signals from the suprachiasmatic nucleus (SCN), the master circadian clock in the hypothalamus. The pineal gland is surrounded by connective tissue and contains specialized cells called pinealocytes, which are responsible for melatonin production (Ahmad *et al.*, 2013). It is synthesized from serotonin, which itself is derived from the amino acid tryptophan. The biosynthetic pathway involves several steps: tryptophan is converted to tryptamine, then to serotonin, followed by N-acetylserotonin, and finally to melatonin (Back, 2016; Sing *et al.*, 2020). The enzyme arylalkylamine N-acetyltransferase (AANAT) plays a crucial role in converting serotonin to N-acetylserotonin (Reiter *et al.*, 2016). It plays a key role in regulating sleep-wake cycles, seasonal behaviours, and circadian rhythm (Megha *et al.*, 2024). Its secretion is inhibited by light and stimulated in darkness, making it a reliable signal of nighttime rather than merely a sleep-inducing hormone (Zisapel, 2018; Pevet *et al.*, 2022). It is released during the night in alignment with the body's internal clock, helping to coordinate physiological processes associated with darkness. Disruption of melatonin rhythms is linked to circadian sleep-wake disorders, characterized by persistent sleep disturbances (Williams *et al.*, 2016). Moreover, recent research suggests that altered circadian rhythms and clock gene expression may contribute to liver conditions such as steatosis, inflammation, and even cancer (Sato *et al.*, 2020). As a chronobiotic agent, externally administered melatonin can help treat circadian misalignment and related disorders (Pfeffer *et al.*, 2018).

1.2 Melatonin Receptors: Structure, Signaling, and Therapeutic Roles

Melatonin acts through specific receptors, MT1 and MT2which are found in various organs including the brain, retina, cardiovascular system, liver, intestines, kidneys, and immune cells (Ekmekcioglu, 2006). MT1 receptors primarily mediate melatonin's effects on the SCN, while MT2 receptors play a secondary role (Waly, 2015). In mammals, melatonin acts through two G-protein-coupled receptor (GPCR) subtypes: MT1 and

MT2 (Jockers *et al.*, 2016). These receptors are primarily located on the plasma membrane and may also be present in mitochondria (Boutin *et al.*, 2020). Since mitochondria are central to redox balance, circadian regulation, and aging, their interaction with melatonin receptors suggests a broader physiological influence (Yanar *et al.*, 2019). Upon activation, MT1 and MT2 receptors initiate various signal transduction pathways, leading to diverse cellular responses depending on the tissue and context (Enderby *et al.*, 2003). Numerous proteins have been identified that interact with these receptors, further expanding their functional complexity (Cecon *et al.*, 2017).

Clinically, melatonin receptors are targeted in the treatment of insomnia, circadian rhythm sleep disorders, and major depressive disorder (Liu *et al.*, 2016). MT2, in particular, has shown therapeutic relevance in managing type 2 diabetes (Wang *et al.*, 2022). Additionally, both receptors are being explored for their roles in cancer and autoimmune diseases (Nikolaev *et al.*, 2022).

1.3 Comparative Significance of Studying Melatonin Across Species

Studying melatonin in different species such as: humans, rats, and fishes offer valuable insights into its pharmacological functions and evolutionary relevance. In fish, melatonin induces pigment aggregation toward the center of pigment cells, including in embryos. This adaptation to dim light results in a paler body appearance, helping the organism blend into its environment (Mubashshir *et al.*, 2023). In rodents, particularly rats and mice, melatonin plays a crucial role in regulating daily locomotor activity. Mice with a fully functional melatonergic system show more precise and consistent movement patterns compared to melatonin-deficient mice or those lacking MT1 and MT2 receptors (Pfeffer *et al.*, 2017). Additionally, melatonin exhibits antioxidative properties that influence reproductive regulation in fish. Current theories on its role in reproduction are largely based on data from mammals and birds, and have been further supported by recent studies in carp (Hasan, 2016).

2. REVIEW LITERATURE

2.1 Previous Studies on Melatonin and Its Receptors

Melatonin exerts its biological effects primarily by activating two G-protein-coupled receptors: MT1 (type 1A) and MT2 (type 1B), which play key roles in regulating the sleep–wake cycle (Stauch *et al.*, 2019). These receptors are encoded by the *MTNR1A* and *MTNR1B* genes, respectively (Jockers *et al.*, 2016).MT2 receptors can also interact with other G-protein-coupled receptors such as GPR61, GPR62, and GPR135. These interactions lead to (i) the formation of receptor heteromers and (ii) inhibition of melatonin-induced β-arrestin2 recruitment to MT2, thereby modulating downstream signaling (Oishi *et al.*, 2017). Melatonin functions as a neurotransmitter, hormone, therapeutic agent, and regulator of various physiological processes. Its effects are primarily mediated through MT1 and MT2 receptors at physiological concentrations (Slominski *et al.*, 2023). Synthetic melatonin analogs- such as ramelteon, tasimelteon, and agomelatine, are used clinically to treat central nervous system (CNS) disorders. These compounds non-selectively activate both MT1 and MT2 receptors (Elisi *et al.*, 2021). Although MT1 and MT2 share similar ligand-binding drug development and G-protein coupling mechanisms (Wang *et al.*, 2022). Both receptors display high genetic similarity and bind melatonin with strong affinity (Jockers *et al.*, 2016). Functionally, MT2 activation enhances the duration and quality of non-REM sleep, while MT1 activation is more effective in stabilizing circadian rhythms (Sanchez *et al.*, 2021). Additionally, melatonin's antioxidant effects are mediated through both MT1 and MT2 receptors. In neurodegenerative conditions, activation of these receptors stimulates the ERK1/2 and PI3K/AKT signaling pathways, promoting neuronal survival and reducing oxidative stress (Cardinali, 2019).Ramelteon (RMT), a potent melatonin receptor agonist, has also been explored in silico for structure-based drug design, aiding the development of novel therapeutic agents (Menezes *et al.*, 2024).Biochemical studies using radioligand binding assays have confirmed melatonin's high-affinity binding to its receptors. This interaction inhibits adenylate cyclase activity, thereby modulating cyclic AMP (cAMP) signaling within target cells (Dubocovich *et al.*, 2010). Further research has shown that melatonin receptor activation induces conformational changes that facilitate coupling with Gi/o proteins, leading to downstream effects on gene transcription and ion channel regulation (Jockers etal., 2016).

Beyond circadian regulation, melatonin's receptor-mediated signaling also contributes to its anti-inflammatory and antioxidant properties, highlighting its broader physiological significance (Cecon *et al.*, 2018).

2.2 In Silico Studies on Melatonin Binding in Mammals and Fish Species

Melatonin (MT), a key endogenous regulator of physiological rhythms, plays a crucial role in both central and peripheral processes. In fish, it supports environmental adaptation by modulating various physiological functions (Kuz'mina, 2020). Among vertebrates, including fish, melatonin also regulates seasonal reproduction (Maitra, 2016).

This versatile hormone influences pigment dispersion in chromatophores by binding to specific receptor subtypes (Mubashshir *et al.*, 2023). In silico approaches such as molecular docking and molecular dynamics (MD) simulations are commonly used to predict melatonin–receptor interactions (Niu *et al.*, 2023).

In teleost fish, melatonin receptor density and binding affinity vary across tissues and are influenced by

circadian rhythms and seasonal changes (Royan *et al.*, 2020). Comparative genomic studies reveal that the mammalian GPR50 receptor shares evolutionary similarity with the fish-specific Mel1c receptor, although GPR50 does not bind melatonin (Cecon *et al.*, 2018). Fish and other non-mammalian vertebrates possess an additional melatonin receptor subtype, Mel1c, which differs functionally from mammalian receptors but is evolutionarily related (Hasan, 2016). In teleosts, melatonin regulates circadian and seasonal rhythms by binding to MT1, MT2, and Mel1c receptors located in the brain, retina, and pineal gland (Royan *et al.*, 2020). Binding studies in species such as goldfish (*Carassius auratus*) demonstrate high receptor affinity and distinct distribution patterns across brain regions. Receptor density and binding affinity in fish are also modulated by environmental factors like temperature and photoperiod, indicating adaptive binding dynamics (Hasan, 2016). Despite the conserved mechanism of melatonin binding across vertebrates, species-specific physiological differences are largely driven by receptor subtype diversity (Liu *et al.*, 2019). Computational modeling and docking studies have elucidated the interaction pathways of melatonin with MT1 and MT2 receptors, highlighting structural features conserved across species (Gerdin *et al.*, 2021). Further research on fish melatonin receptors shows similar binding orientations and conserved amino acid residues, suggesting strong ancestral preservation of receptor structure and function (Falcón *et al.*, 2020).

2.3 Comparative and Phylogenetic Analyses of Melatonin Receptors in Mammals and Fish

Phylogenetic studies indicate that melatonin receptors in vertebrates originated from multiple ancestral genes. Over time, these receptors evolved differently across species. In teleost fish, whole-genome duplication events led to the emergence of additional receptor variants, such as MT1a/MT1b and MT2a/MT2b. In contrast, mammals and birds lost certain subtypes, including Mel1d (Denker *et al.*, 2019). Humans and rats primarily retain only MT1 and MT2 receptors, which are mainly involved in circadian regulation. Teleost fish, however, possess extra paralogs that show broader tissue distribution and more diverse signaling functions (Maugars, 2020).

Structural and evolutionary analyses reveal that mammalian melatonin receptors are more conserved, supporting core metabolic and circadian roles. In contrast, teleost receptor paralogs have adapted to perform specialized functions in different tissues (Stauch, 2020).

3. MATERIALS AND METHODS

3.1 Identification and retrieval of protein sequences

The National Center for Biotechnology Information (NCBI) is part of the National Library of Medicine (NLM) under the National Institutes of Health (NIH). Its goal is to create and maintain advanced information systems that support research in molecular biology, established in 1988(Eric W Sayers *et al.*, 2025). The National Center for Biotechnology Information (NCBI) hosts 31 distinct repositories and knowledgebases, collectively comprising approximately 4.6 billion records. Most of these data resources can be accessed via the Entrez search system at https://www.ncbi.nlm.nih.gov/search (Eric W Sayers *et al.*, 2022). Retrieve all protein sequences of melatonin from NCBI in FASTA format for humans (*Homo sapiens)*, rats (*Rattus norvegicus)*, and fish (*Labeo rohita*). The complete methodology is shown in Table1 provides a sketch of the many bioinformatics tools and databases used in the current study.

3.2 Proteins interaction and network analysis

The STRING database (Search Tool for the Retrieval of Interacting Genes/Proteins) provides global insights into functional protein–protein interactions. The STRING database (http://string-db.org; Szklarczyk *et al.*, 2015) integrates and evaluates protein–protein interactions, including both direct (physical) and indirect (functional) associations. Version 10.0 expands coverage to over 2,000 organisms, requiring scalable algorithms to transfer interaction data across species, including mammals and fish.

3.3 3D Structure Prediction

Phyre2.2, an updated version of the widely used template modelling portal Phyre2, featuring several key enhancements. The key advancement allows users to submit theirsequence, after which Phyre2.2 selects the most appropriate AlphaFold model as a structural template.Template-based modelling, or homology modelling, is a reliable method for predicting protein structures from amino acid sequences. This approach will be applied in our study to melatonin receptors from mammals (humans and rats) and fish**.** Phyre2.2 is freely accessible to all users, including those in commercial settings, at https://www.sbg.bio.ic.ac.uk/phyre2/; Powell *et al.*, 2025).

3.4 3D structure Visualization and Result analysis

Biovia Discovery Studio (BDS) is a comprehensive visualization platform designed to interpret and communicate molecular simulation and analysis results effectively. It is support researchers in interpreting and presenting results from molecular simulations, docking studies, and related analyses.This software provides guidance for visualizing molecular structures in 3D of mammals (human, rat) and fish, identifying key protein-ligand interactions, including hydrogen bonds, hydrophobic contacts, aromatic stacking, and ionizable regions. In addition, the software includes visualization features for generating publication-quality molecular images. In addition, the software includes visualization features for generating publication-quality molecular images at BIOVIA Discovery Visualizer7: https://discover.3ds.com/discovery-studio-visualizer-download (Umi Baroroh *et al.*, 2023).

Table 1. The Various software's and database used in current study

S.NO.	Software's/Databases	Link
1	National Center for Biotechnology Information (NCBI)	https://www.ncbi.nlm.nih.gov
2	Search Tool for the Retrieval of Interacting Genes/Proteins (STRING)	http://string-db.org
3	Protein Homology/Analogy Recognition Engine version 2 (Phyre2)	https://www.sbg.bio.ic.ac.uk/phyre2/
4	Biovia Discovery Studio (BDS)	https://discover.3ds.com/discovery-studio-visualizer-download

4. RESULTS AND DISCUSSION

4.1 Identification and retrieval of Melatonin protein sequences of mammals (Human, Rat) and fish

Melatonin protein sequences from fish and mammals (human and rat) were identified and retrieved in FASTA format from the NCBI database. All retrieved melatonin protein sequences are listed below, each is identified by a FASTA header marked with the symbol ">". Identifying and retrieving melatonin protein sequences in mammals (such as humans and rats) and fish requires an understanding of the genetic and protein components involved in melatonin signaling and synthesis. This knowledge also supports the analysis of protein interactions and the prediction of melatonin three-dimensional structure. Three melatonin receptor genes have been cloned: MT1, also known as Mel1a or MTNR1A; MT2 (Mel1b or MTNR1B); and Mel1c (MTNR1C). MT1 and MT2 are found in humans and other mammals, while Mel1c is an additional subtype identified in fish(Li *et al.*, 2013)suggests a more intricate signaling system than that observed in mammals(Sakai *et al.*, 2019).Melatonin receptors, which belong to the G protein-coupled receptor family, play key roles in mediating melatonin's physiological effects. Their sequences can be obtained through genomic and transcriptomic analyses. Additionally, the melatonin biosynthesis pathway particularly the enzyme arylalkylamine N-acetyltransferase (AANAT)offers valuable information about proteins associated with melatonin production across these species. While most vertebrate groups possess only a single aanat gene, studies have indicated that fish exhibit the greatest diversity, with three distinct aanat genes (aanat1a, aanat1b, and aanat2). These variants are thought to support specialized functions such as seasonal migration, amphibious aerial vision, and adaptation to cave or deep-sea environments (Huang *et al.*, 2022). All retrieved melatonin protein sequences included in this study support comparative analyses between mammals (human and rat) and fish.

4.2 Protein interaction and network analysis of melatonin in mammals (Human, Rat) and fish

The availability of retrieved melatonin protein sequences offers a valuable dataset for analyzing protein interactions and receptor network dynamics in mammals (human and rat) and fish.

4.2.1 The protein interaction network of melatonin receptors MT1A and MT1B in human (*Homo sapiens*) and their network status

The STRING network analysis of MT1A and MT1B melatonin receptors in Human (*Homo sapiens)* reveals a central cluster of interacting proteins, including G protein subunits such as GNGT1, GNGT2, GNG2, GNG3, GNG5, GNG7, GNG8, GNG10, GNG11, GNG12, and GNG13, as well as GNB1, GNB3, GNB4, and GNB5. Peripheral interactions involve proteins such as HTR1D, HTR7, HTR1F, KCNJ3, PTGER3, PREX1, RRH, GIPR, ADCY5, GPR18, PER3, and SUCNR1, indicating broader signaling connectivity and functional diversity. It offers detailed network statistics for each protein–protein interaction, including interaction strength, confidence scores, number of nodes and edges, node degree, average local clustering coefficient, and associated functional relationships (Fig. 1).

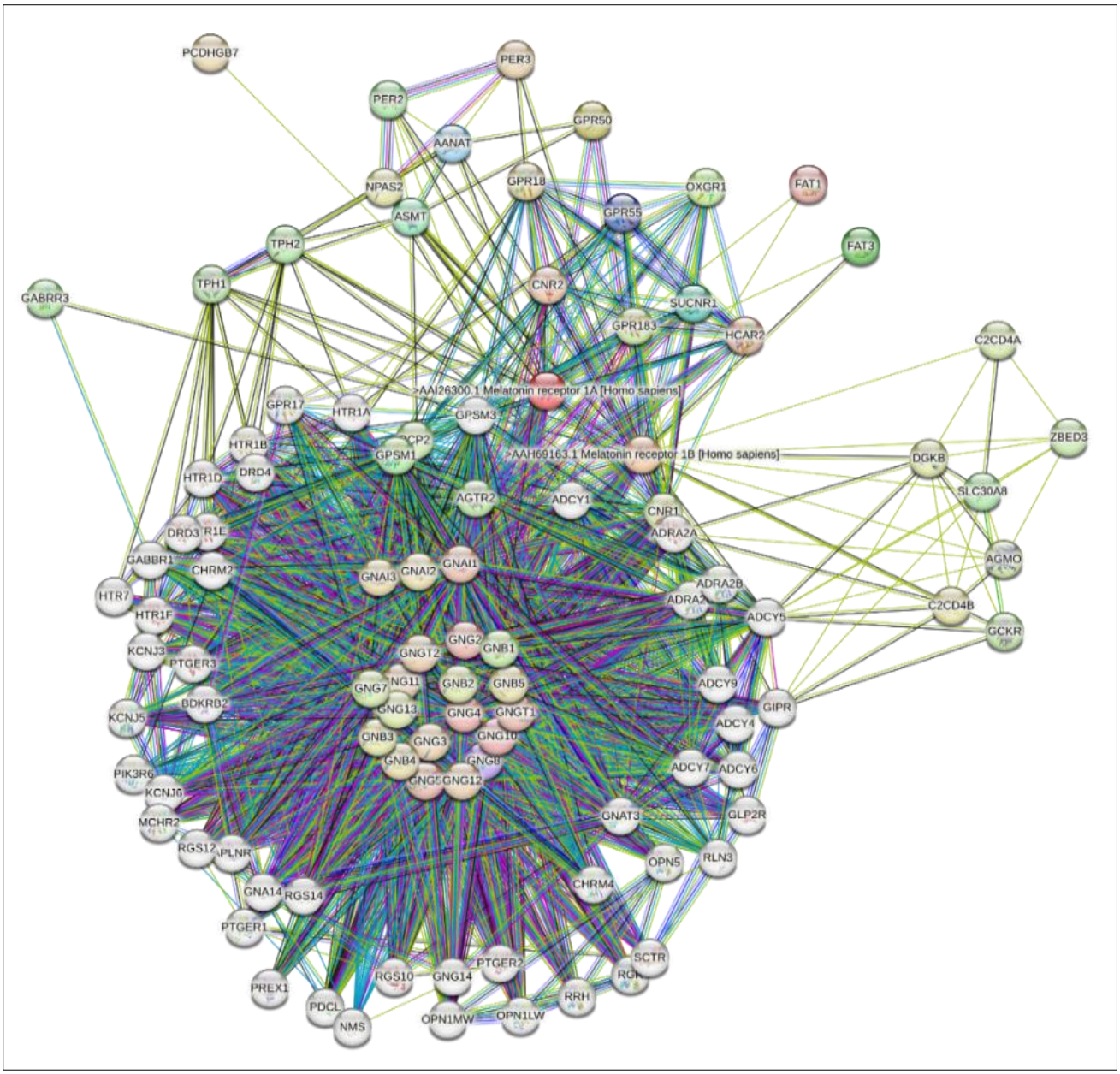

Fig 1. Protein-protein interaction and network stat of Human (*Homo sapiens)* Melatonin 1A (MT1A) (protein sequence id >AAI26300.1) and Melatonin 1B (MT1B) (protein sequence id >AAH69163.1) receptor by STRING database number of nodes: 102, number of edges: 1687, average node degree: 33.1, avg. local clustering coefficient: 0.782, expected number of edges: 166, PPI enrichment p-value: < 1.0e-16.

4.2.2 The protein interaction network of melatonin receptors MT1A and MT1B in Rat (*Rattus norvegicus*) and their network status

STRING network analysis of MT1A and MT1B melatonin receptors in rats (*Rattus norvegicus*) shows a central cluster of proteins that interact with each other. Most of these proteins are G protein subunits, such as GNB1, GNB2, GNB3, GNG2, GNG3, GNG4, GNG7, GNG10, GNG12, GNG13, GNG14, GNAQ, GNAI1, GNAI3, GNGT1, and GNGT2. Peripheral interactions include proteins like OPRM1, KCNJ3, ADCY3, CDC123, AANAT, AWAT2, and CHRM2, indicating broad signaling connectivity and functional diversity. Additionally, one protein identified by the Ensembl ID ENSRNOP00000057378 appears in the network, although its functional annotation remains unavailable, highlighting a potential target for further characterization (Fig. 2).

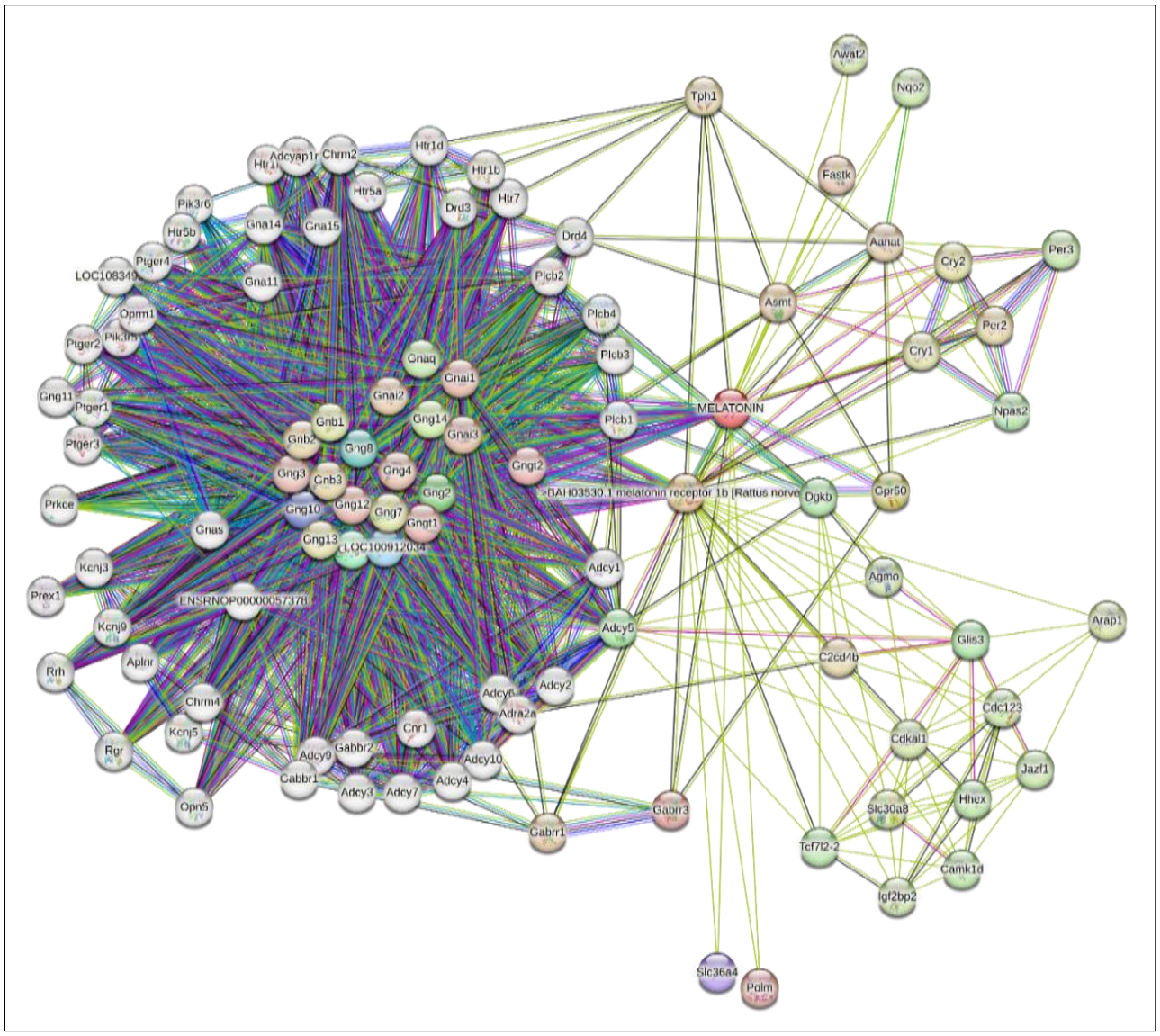

Fig. 2: Protein-protein interaction and network stat network of Rat (*Rattus Norvegicus)* Melatonin 1A (MT1A) (protein sequence id>NP_446128.1) and Melatonin 1B (MT1B) (protein sequence id>BAH03530.1) receptor by STRING database number of nodes: 102, number of edges: 1496, average node degree: 29.3 avg. local clustering coefficient: 0.772, expected number of edges: 155, PPI enrichment p-value: < 1.0e-16

4.2.3 The protein interaction network of melatonin receptors MT1A, MT1B and MT1C in Fish (*Labeo rohita*) and their network status

STRING network analysis of MT1A, MT1B, and MT1C melatonin receptors in *Labeo rohita* reveals a central group of interacting proteins, including ROHU_035712, ROHU_031043, ROHU_015480, ROHU_018781, and ROHU_012079. Additional peripheral proteins—such as ROHU_035640, ROHU_002031, ROHU_005549, ROHU_022568, ROHU_008322, and ROHU_018698further expand the network, indicating broad signaling connectivity and functional diversity within the melatonin receptor system (Fig. 3).

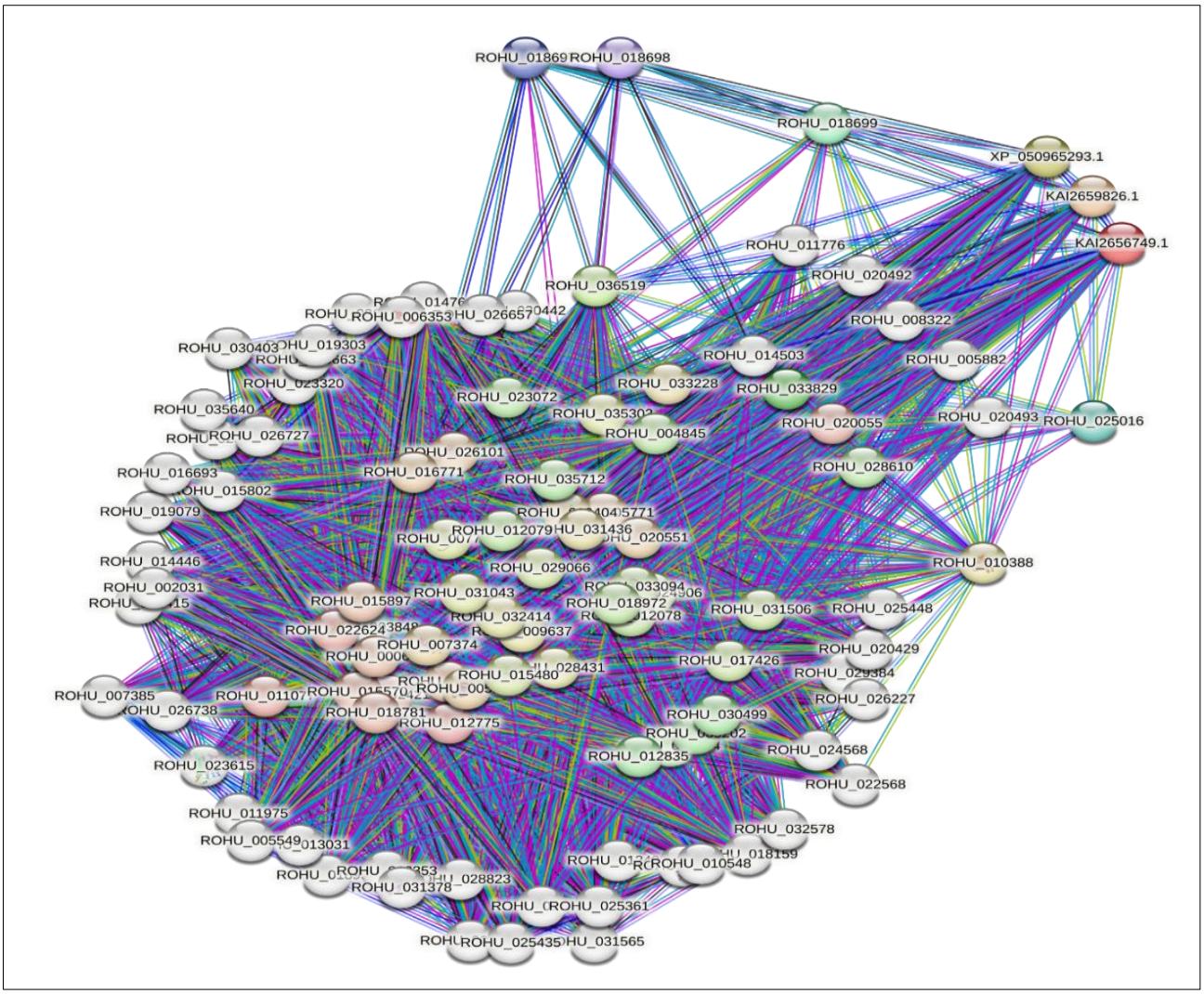

Fig 3. Protein-protein interaction and network stat network of Fish (*Labeo rohita)* Melatonin 1A (MT1A) (protein sequence id>KAI2656749.1), Melatonin 1B (MT1B) (protein sequence id>KAI2659826.1 and Melatonin 1C (MT1C) (protein sequence id>XP_050965293.1) receptor by STRING database number of nodes: 103, number of edges: 2907, average node degree: 56.4 avg. local clustering coefficient:

0.55, expected number of edges: 166, PPI enrichment p-value: < 1.0e-16

Protein–protein interaction (PPI) studies have shown that melatonin receptors can form both homodimers and heterodimers, which affect their sensitivity, downstream signaling pathways, and cellular localization. Methods such as bioluminescence resonance energy transfer (BRET) and co-immunoprecipitation have been used to study these interactions, revealing dynamic crosstalk between receptors and regulation by associated proteins(Oishi & Jockers, 2022). Network analysis using bioinformatics tools such as STRING allows visualization of protein networks associated with melatonin receptors. These analyses identify key interaction partners-such as G proteins, arrestins, and kinases that regulate receptor desensitization, internalization, and signal transduction. Comparative mapping between mammals and fish reveals conserved core interactions as well as species-specific components, highlighting evolutionary adaptations and functional diversity(Cecon *et al.*, 2023; Okamoto *et al.*, 2024). Integrating receptor PPI data with biosynthetic enzyme networks provides a holistic view of melatonin role in vertebrate physiology.

4.3 3D Structure Prediction of melatonin in mammals (Human, Rat) and fish

The 3D structure of melatonin receptors in mammals (human, rat) and fish was predicted using homology modeling and surface shape analysis, which are useful for protein docking studies.This approach relies on comparing protein profiles and predicting secondary structures, allowing accurate model generation even when the target sequence has low similarity to known protein structures.

4.3.1 3DStructural Features and Comparative Insightsof melatonin receptors

All predicted models showed the typical 7-transmembrane helix structure found in GPCRs. The human and rat melatonin receptors were structurally very similar, while fish receptors showed slight shifts in helix

orientation, which may reflect evolutionary changes.A key amino acid, Asn195 (based on human MT1 receptor numbering), was conserved across humans, rats, and fish, suggesting that melatonin binds in a similar way in all three species. However, fish receptors had more flexible loop regions near the binding site, which could affect how strongly melatonin binds and how the receptor responds (Wang *et al.,* 2022). Structural differences in the third intracellular loop and the C-terminal tail may influence how these receptors interact with G-proteins. In mammals, melatonin receptors mainly couple with Gi/o proteins, while in fish, they may activate different signaling pathways depending on their structure.Understanding the 3D structure of melatonin receptors is important for studying how they bind to melatonin, how they become activated, and how their function differs between species. Since there are few experimentally determined structures, computational tools like Phyre2 are valuable for predicting their shape and guiding further research(Kelley *et al.,* 2015). Studying these structural features helps improve drug development aimed at melatonin receptors, which are involved in treating sleep problems, mood disorders, and bone-related diseases. It also deepens our understanding of how these receptors have evolved in different vertebrate species (Fig. 4).

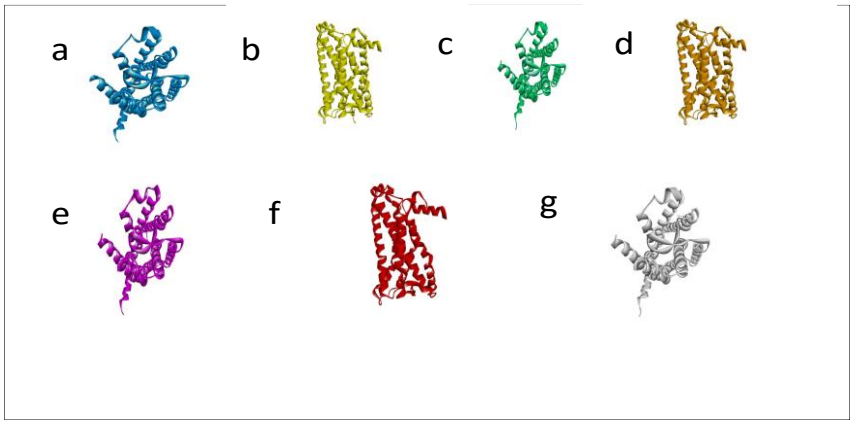

Fig. 4. Overall 3D Structure of melatonin receptors inHuman *(Homo sapience)*:Melatonin 1A (hMT1A-Blue) (protein sequence id >AAI26300.1), Melatonin 1B (hMT1B-Yellow) (protein sequence id >AAH69163.1); In Rat *(Rattus norvegicus)*:Melatonin 1A(rMT1A-Green) (protein sequence id>NP_446128.1), Melatonin 1B (rMT1B-Burnt orange)(protein sequence id>BAH03530.1); In Fish *(Labeo rohita)*:Melatonin 1A (fMT1A-Purple)(protein sequence id>KAI2656749.1), Melatonin 1B (fMT1B-Red)(protein sequence id>KAI2659826.1) and Melatonin 1C (fMT1C-White) (protein sequence id>XP_050965293.1).

4.3.2Structural Characteristics and Comparative Analysis of 3D Surface Morphology

We examine the 3D surface structure of melatonin receptors in humans, rats, and selected fish species using computational modeling and docking simulations to identify structural similarities and differences that affect how ligands bind. The differences in surface structure and loop flexibility seen in fish receptors may represent adaptations to varying light environments and specific physiological needs.

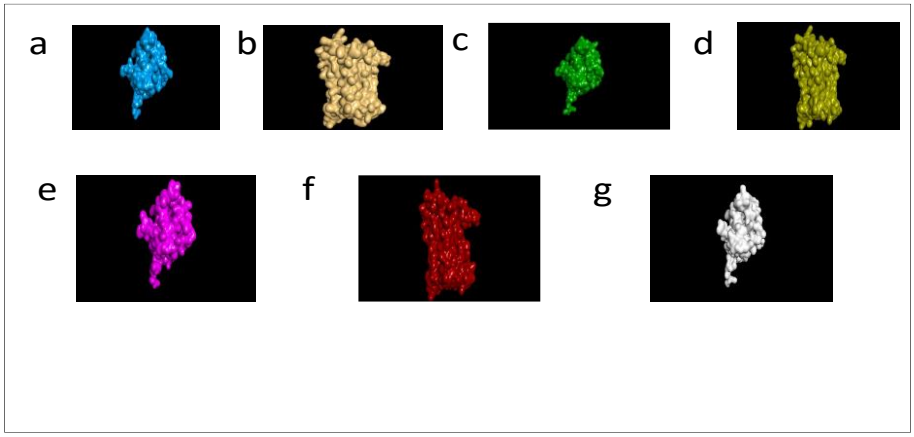

Fig. 5: Overall 3D Surface morphology of melatonin receptors in Human *(Homo sapience)*:Melatonin 1A (hMT1A-Blue) (protein sequence id >AAI26300.1), Melatonin 1B (hMT1B-Yellow) (protein sequence id >AAH69163.1); In Rat *(Rattus norvegicus)*:Melatonin 1A(rMT1A-Green) (protein sequence id>NP_446128.1), Melatonin 1B (rMT1B-Burnt orange) (protein sequence id>BAH03530.1); In Fish *(Labeo rohita)*:Melatonin 1A (fMT1A-Purple)(protein sequence id>KAI2656749.1), Melatonin 1B (fMT1B-Red)(protein sequence id>KAI2659826.1) and Melatonin 1C (fMT1C-White) (protein sequence id>XP_050965293.1)

Surface analysis showed that melatonin receptors in humans and rats have a compact and hydrophobic binding pocket, which is well-suited for strong melatonin binding. The extracellular loops of MT1 and MT2 help determine ligand specificity and support receptor activation. In comparison, fish melatonin receptors have more varied surface structures. For example, the Mel1c receptor in fish has a wider and more exposed binding cavity, which may allow it to interact with a broader range of ligands. Electrostatic surface mapping revealed that fish receptors have more negatively charged residues near the binding site, which could influence how ligands are positioned and how strongly they bind. Docking studies using AutoDock Vina confirmed that melatonin binds effectively to all receptor types, although there are small differences in binding energy and orientation. In mammals, melatonin forms stable hydrogen bonds with conserved residues like Asn175 and Phe192, while in fish, different residues such as Ser117 and Tyr281 help stabilize the ligand(Mubashshir *et al.*, 2023).

4.4 3D structure Visualization results in Comparative manner

We focused on visualizing the 3D structures of melatonin receptors in mammals (humans and rats) and fish, specifically examining aromatic interactions, hydrogen bonding, and ionizability. These features were analyzed to understand how structural differences influence ligand binding and receptor function across species.

4.4.1 Aromatic Interaction Result

Aromatic interactions are important for stabilizing ligand-receptor complexes, particularly in GPCRs such as melatonin receptors. These interactions usually occur through π–π stacking or edge-to-face contacts between aromatic amino acids in the receptor and the aromatic ring of the ligand (Fig. 6).

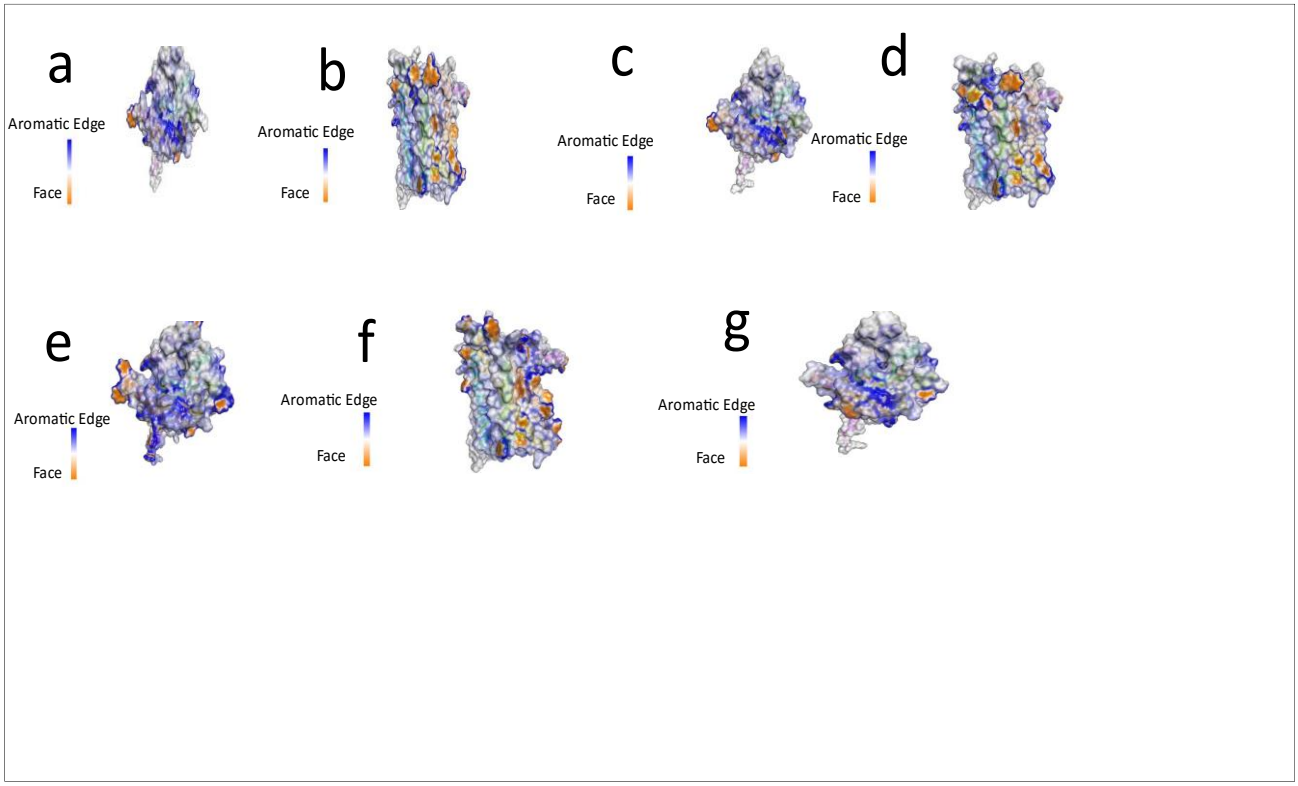

Fig. 6: Overall 3D Aromatic Interaction Result of melatonin receptors in Human *(Homo sapience)*:Melatonin 1A (hMT1A) (protein sequence id >AAI26300.1), Melatonin 1B (hMT1B) (protein sequence id >AAH69163.1); In Rat *(Rattus norvegicus)*:Melatonin 1A(rMT1A) (protein sequence id>NP_446128.1), Melatonin 1B (rMT1B) (protein sequence id>BAH03530.1); In Fish *(Labeo rohita)*:Melatonin 1A (fMT1A)(protein sequence id>KAI2656749.1), Melatonin 1B (fMT1B)(protein sequence id>KAI2659826.1) and Melatonin 1C (fMT1C) (protein sequence id>XP_050965293.1).The color code is as follows: Blue colour denote is Aromatic edge and orange colour denote is Face. In MT1 and MT2 receptors, aromatic amino acids such as Phe192, Tyr281, and Trp251 are located near the ligand-binding site. These residues form π–π stacking interactions with the indole ring of melatonin, which helps stabilize the ligand and supports strong binding. Structural visualization reveals a compact cluster of aromatic residues, especially in transmembrane domains TM5 and TM6, creating a hydrophobic environment that limits solvent interference and enhances binding specificity. In contrast, Mel1c and related fish receptor subtypes contain aromatic residues like Tyr117 and Phe204, but these are more widely spaced. The binding pocket in these receptors is broader and more exposed to the solvent, which may reduce the strength of π–π stacking but allows for alternative aromatic interactions. This more flexible arrangement may reflect an evolutionary adaptation to diverse light environments in aquatic habitats, enabling broader ligand recognition(Mubashshir *et al.*, 2023; Wang *et al.*, 2022)

4.4.2 H-Bonding Result

Hydrogen bonds play an essential role in positioning the ligand correctly, activating the receptor, and ensuring stable binding. These bonds typically form between polar amino acid residues in the receptor and specific functional groups on the melatonin molecule (Fig. 7).

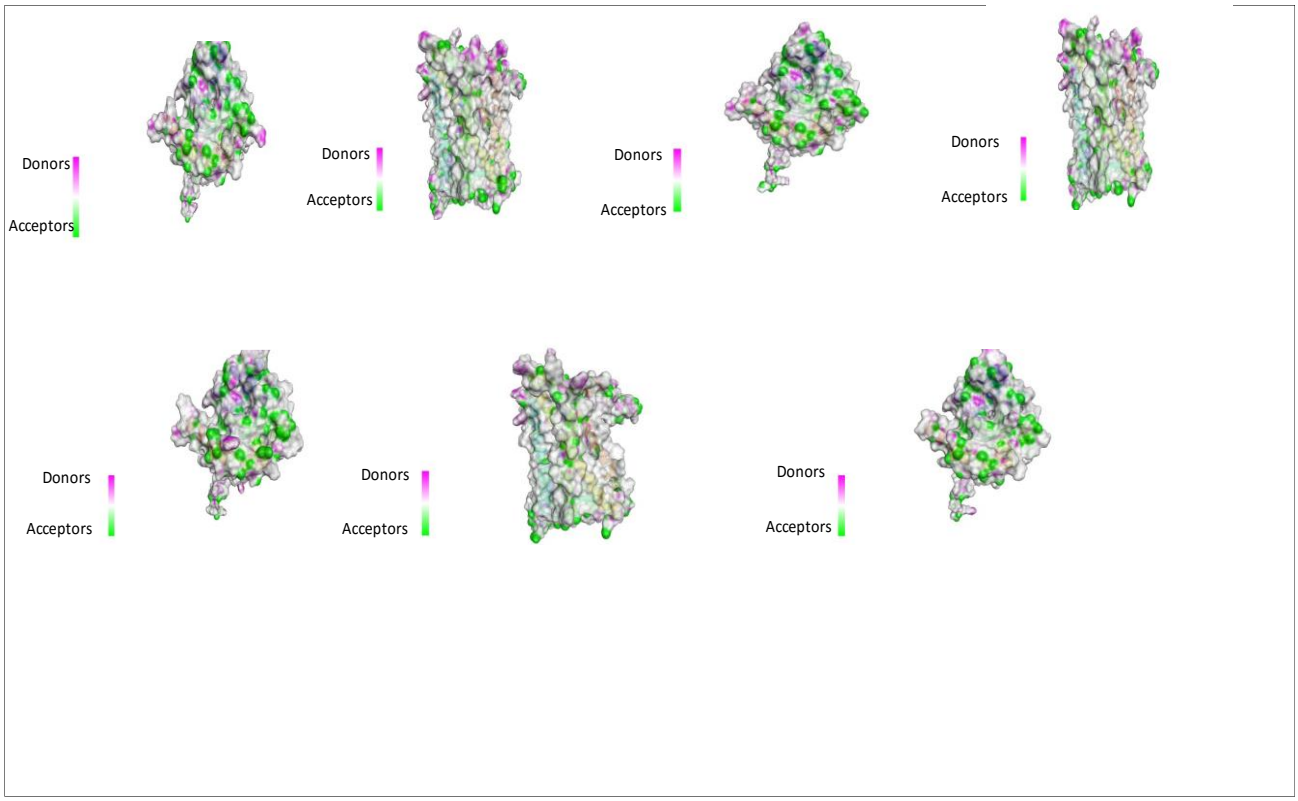

Fig. 7: Overall 3D H-Bonding Result of melatonin receptors in Human *(Homo sapience)*: Melatonin 1A (hMT1A) (protein sequence id >AAI26300.1), Melatonin 1B (hMT1B) (protein sequence id >AAH69163.1); In Rat *(Rattus norvegicus)*:Melatonin 1A (rMT1A) (protein sequence id>NP_446128.1), Melatonin 1B (rMT1B) (protein sequence id>BAH03530.1); In Fish *(Labeo rohita)*: Melatonin 1A (fMT1A) (protein sequence id>KAI2656749.1), Melatonin 1B (fMT1B) (protein sequence id>KAI2659826.1) and Melatonin 1C (fMT1C) (protein sequence id>XP_050965293.1). The color code is as follows: Pink colour denote is Donors and Green colour denote is Acceptors

In mammalian melatonin receptors, key residues such as Asn175, Ser110, and His195 form stable hydrogen bonds with the methoxy and amide groups of melatonin. These bonds are located deep within the binding pocket, helping to position the ligand accurately. Structural visualization shows consistent hydrogen bond geometry, with optimal donor-acceptor distances ranging from approximately 2.8 to 3.2 Å. This hydrogen bonding network is conserved across species, suggesting strong evolutionary pressure to maintain effective ligand binding(Barrett, 2004; López Sastre *et al.*, 2001). In fish receptors like those in Labeo rohita, residues such as Ser117, Thr203, and Tyr281 also contribute to hydrogen bonding, but with more variation in bond angles and distances. The binding pocket in fish is more flexible, allowing multiple bonding configurations. Electrostatic surface mapping reveals higher polarity around the binding site, which may support transient hydrogen bond formation(Sahoo, 2013). Overall, mammalian receptors show a rigid and conserved hydrogen bonding pattern that supports high-affinity binding, while fish receptors provide a more dynamic environment that may accommodate a wider range of ligands. These differences reflect functional divergence in melatonin signaling between terrestrial and aquatic vertebrates.

4.4.3 Ionizability:

Ionizability refers to the ability of receptor residues to gain or lose protons, influencing charge distribution and ligand interaction. It is closely tied to the receptor's electrostatic surface (Fig. 8).

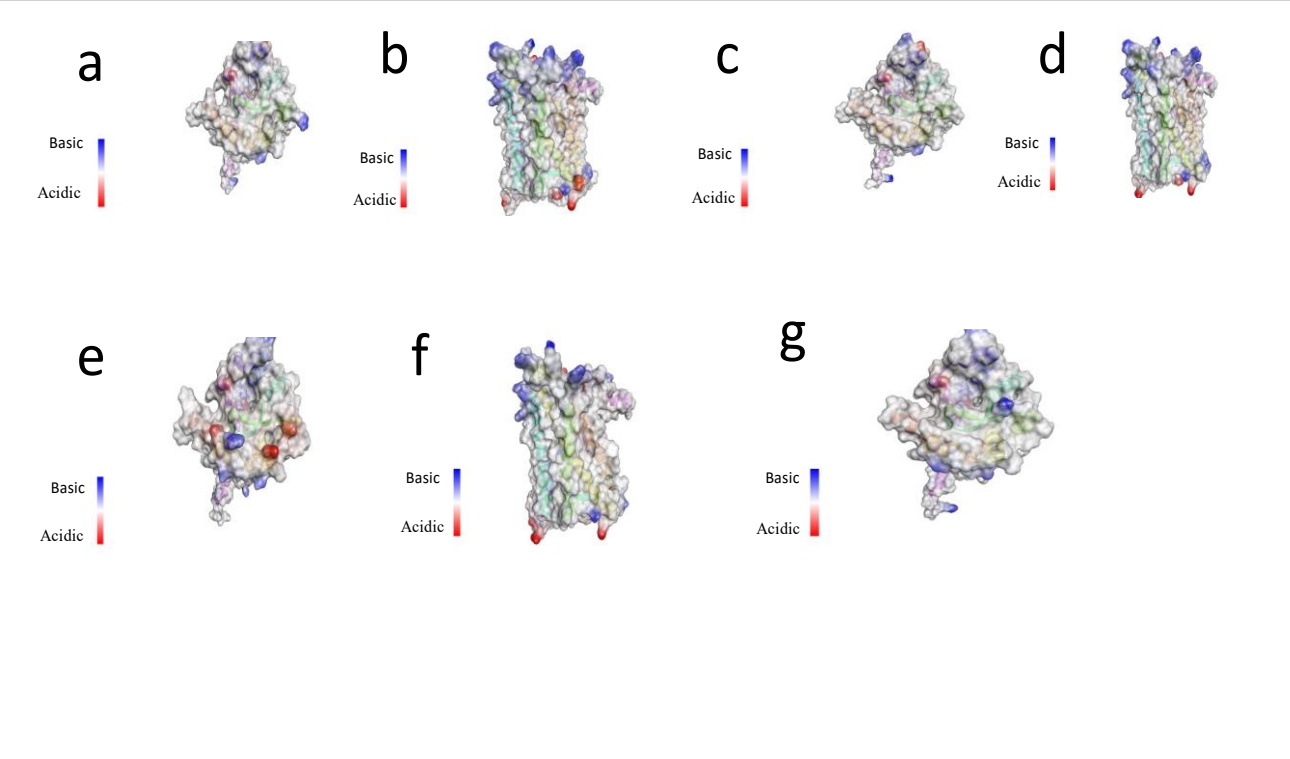

Fig. 8: Overall 3D Ionizability Result of melatonin receptors in Human *(Homo sapience)*:

Melatonin 1A (hMT1A) (protein sequence id >AAI26300.1), Melatonin 1B (hMT1B) (protein sequence id >AAH69163.1); In Rat *(Rattus norvegicus)*:Melatonin 1A (rMT1A) (protein sequence id>NP_446128.1), Melatonin 1B (rMT1B) (protein sequence id>BAH03530.1); In Fish *(Labeo rohita)*: Melatonin 1A (fMT1A) (protein sequence id>KAI2656749.1), Melatonin 1B (fMT1B) (protein sequence id>KAI2659826.1) and Melatonin 1C (fMT1C) (protein sequence id>XP_050965293.1). The color code is as follows: Blue colour denote is Basic and Red colour denote is Acidic. In mammalian melatonin receptors, the binding pocket is generally neutral to mildly hydrophobic, with few ionizable residues. Amino acids such as Asp120 and Glu196 create localized negative charges that help guide melatonin into the pocket. Electrostatic surface maps show a balanced charge distribution, which reduces repulsion and supports strong ligand binding. This ionizability profile is well-suited for stable docking under physiological pH conditions(https://molsoft.com/icm_browser.html). In fish receptors, such as those in Labeo rohita, the binding site contains more negatively charged residues, including Glu117 and Asp203. This may reflect an adaptation to fluctuating aquatic pH levels and the presence of melatonin analogs with different charge properties. The receptor surface is more polar, increasing ionizability and potentially influencing how the ligand is oriented during binding. Visualization of the electrostatic surface reveals broader charge gradients, suggesting that fish receptors may be more responsive to environmental changes(Dhandare *et al.*, 2022). Comparative analysis shows that mammalian receptors maintain low ionizability and stable charge environments, which support precise and consistent signaling. In contrast, fish receptors exhibit higher ionizability and greater flexibility in ligand interactions, allowing them to function effectively under diverse physiological conditions. These differences highlight species-specific adaptations in melatonin receptor structure and function.

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